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(54) Title: COSMETIC METHOD OF TREATING SKIN

(57) Abstract: A cosmetic method for treating aged, sensitive, dry, flaky, wrinkled and/or photodamaged skin is provided through topical application of a composition which comprises pinolenic acid and/or derivatives thereof. The invention also relates to compositions suitable for such cosmetic treatment.

COSMETIC METHOD OF TREATING SKIN

This invention relates to a cosmetic method of improving the condition and appearance of skin and to the use of pinolenic acid in the preparation of topical compositions for improving the condition and appearance of skin.

Skin is subject to deterioration through dermatological disorders, environmental abuse (wind, air conditioning, central heating) or through the normal aging process (chronoaging) which may be accelerated by exposure of skin to sun (photoaging). In recent years the demand for cosmetic compositions and cosmetic methods for improving the appearance and condition of skin has grown enormously.

Consumers are increasingly seeking "anti-aging" cosmetic products which treat or delay the visible signs of chronoaging and photoaging skin such as wrinkles, lines, sagging, hyperpigmentation and age spots.

Consumers also frequently seek other benefits from cosmetic products in addition to anti-aging. The concept of "sensitive skin" has also raised the consumer demand for cosmetic products which improve the appearance and condition of sensitive, dry and/or flaky skin and to soothe red, and/or irritated skin. Consumers also desire cosmetic products which treat spots, pimples, blemishes etc.

The use of fatty acids, including pinolenic acid, in cosmetic formulations for treating the body is known. For example, it is known (from e.g. WO98/43513) that pine nut oil (which contains pinolenic acid) can have an anti-

inflammatory effect, where it is used to coat nail files to inhibit any infections caused on use of the files.

We have now surprisingly found further undisclosed properties of pinolenic acid, which are useful in cosmetic compositions for topical application to skin to provide previously undisclosed skin care benefits.

We have now found that effective treatment and prevention of normal skin conditions due to chronoaging or photoaging, such as wrinkles, lines, sagging, hyperpigmentation and age spots, may be obtained through the application of cosmetic compositions to the skin which comprise pinolenic acid or derivatives thereof. We have also found that the use of pinolenic acid in cosmetic compositions advantageously provides further skin benefits in addition to anti-aging such as for soothing sensitive and/or irritated skin, improved resilience, reduced dryness/flakiness and reduced hyperproliferaton.

Thus, according to a first aspect of the invention, there is provided a topical composition for application to the human skin comprising an effective amount of pinolenic acid.

According to a further aspect of the present invention there is provided a cosmetic method of providing at least one skin care benefit selected from: treating/preventing wrinkling, sagging, aged and/or photodamaged skin; boosting collagen deposition in skin, boosting decorin production in skin, enhancing tissue repair; improving skin condition and resilience through enhanced barrier formation; treating dry and flaky skin; reduced hyperproliferation; soothing irritated, red and/or sensitive skin; and improving skin texture, smoothness and/or firmness; the method comprising

applying to the skin a topical composition comprising pinolenic acid and/or derivatives thereof.

The present invention also encompasses the use of pinolenic acid and/or derivatives thereof in a topical composition for providing at least one skin care benefit selected from treating/preventing wrinkling, sagging, aged and/or photodamaged skin; boosting collagen deposition in skin, boosting decorin production in skin, enhancing tissue repair; improving skin condition and resilience through enhanced barrier formation; treating dry and flaky skin; reduced hyperproliferation; soothing irritated, red and/or sensitive skin; improving skin texture, smoothness and/or firmness.

The inventive methods and use of pinolenic acid thus provide anti-aging benefits which result in the promotion of smooth and supple skin with improved elasticity and a reduced or delayed appearance of wrinkles and aged skin, with improved skin colour. A general improvement in the appearance, texture and condition, in particular with respect to the radiance, clarity, and general youthful appearance of skin may be achieved. The inventive methods and uses are also beneficial for soothing and calming sensitive and/or irritated skin. Thus the inventive methods advantageously provide a wide range of skin care benefits.

The term "treating" as used herein includes within its scope reducing, delaying and/or preventing the above mentioned skin conditions such as wrinkled, aged, photodamaged, and/or irritated skin and generally enhancing the quality of skin and improving its appearance and texture by preventing or reducing wrinkling and increasing flexibility, firmness, smoothness, suppleness and elasticity of the skin. The

cosmetic methods and the uses of pinolenic acid and/or derivatives according to the invention may be useful for treating skin which is already in a wrinkled, aged, photo-damaged, dry and irritated condition or for treating youthful skin to prevent or reduce those aforementioned deteriorative changes due to the normal aging/photoaging process.

Pinoleic acid is an unsaturated long chain (C18) fatty acid, having three double bonds at the 5,9, and 12 positions. It may be found in e.g. pine nut oil at levels of around 25%.

The invention also includes derivatives of the free acid which thus comprise pinolenic acid moieties. Preferable derivatives include those derived from substitution of the carboxyl group of the acid, such as esters (eg retinyl esters, triglyceride esters, monoglyceride esters, diglyceride esters, phosphoesters), amides (eg ceramide derivatives), salts (eg alkali metal and alkali earth metal salts, ammonium salts); and/or those derived from substitution of the C18 carbon chain, such as alpha hydroxy and/or beta hydroxy derivatives.

In the case of triglyceride ester derivatives, all positional isomers of pinolenic acid substituents on the glycerol backbone are included. The triglycerides must contain at least one pinolenic acid moiety. For example, of the three esterifiable positions on the glycerol backbone, the 1 and 2 positions may be esterified with pinolenic acid and by another lipid at position 3 or as an alternative, the glycerol backbone could be esterified by PA at the 1 and 3 positions with another lipid at position 2.

Oils that may be rich in pinolenic acid triglyceride would thus also suitable for use in the present invention.

Wherever the term "pinolenic" is used in this specification it is to be understood that the derivatives thereof comprising pinolenic moieties are also included. "pinolenic acid moieties" refers to pinolenic fatty acyl portion(s) of a pinolenic derivative.

The active, pinolenic acid, to be employed in accordance with the present invention is present in the topical composition in an effective amount. Normally the total amount of the active is present in an amount between 0.0001% and 50% by weight of the composition. More preferably the amount is from 0.01% to 10% and most preferably from 0.1% to 5% in order to maximise benefits at a minimum cost.

The composition used according to the invention also comprises a dermatologically/cosmetically acceptable vehicle to act as a dilutant, dispersant or carrier for the active pinolenic acid or its derivative. The vehicle may comprise materials commonly employed in skin care products such as water, liquid or solid emollients, silicone oils, emulsifiers, solvents, humectants, thickeners, powders, propellants and the like.

The vehicle will usually form from 5% to 99.9%, preferably from 25% to 80% by weight of the composition, and can, in the absence of other cosmetic adjuncts, form the balance of the composition.

Besides the active, pinolenic acid, other specific skin-benefit actives such as sunscreens, other skin lightening agents, skin tanning agents may also be included. The

vehicle may also further include adjuncts such as perfumes, opacifiers, preservatives, colourants and buffers.

To prepare the topical composition used in the method of the present invention, the usual manner for preparing skin care products may be employed. The active components are generally incorporated in a dermatologically acceptable carrier in conventional manner. The active components can suitably first be dissolved or dispersed in a portion of the water or another solvent or liquid to be incorporated in the composition. The preferred compositions are oil-in-water or water-in-oil emulsions.

The composition may be in the form of conventional skin-care products such as a cream, gel or lotion or the like. The composition can also be in the form of a so-called "wash-off" product e.g. a bath or shower gel, possibly containing a delivery system for the actives to promote adherence to the skin during rinsing. Most preferably the product is a "leave-on" product; a product to be applied to the skin without a deliberate rinsing step soon after its application to the skin.

The composition may packaged in any suitable manner such as in a jar, a bottle, tube, roll-ball, or the like, in the conventional manner.

The method of the present invention may be carried out one or more times daily to the skin which requires treatment. The improvement in skin appearance will usually become visible after 3 to 6 months, depending on skin condition, the concentration of the active components used in the inventive method, the amount of composition used and the frequency with which it is applied. In general, a small

quantity of the composition, for example from 0.1 to 5 ml is applied to the skin from a suitable container or applicator and spread over and/or rubbed into the skin using the hands or fingers or a suitable device. A rinsing step may optionally follow depending on whether the composition is formulated as a "leave-on" or a "rinse-off" product.

In order that the present invention may be more readily understood, the following examples are given, by way of illustration only.

The invention will now be explained by way of example only with reference to the accompanying figures, in which;

- Figures 1 and 2 show the effect of pinolenic acid on decorin and procollagen I upregulation;
- Figures 3 and 4 demonstrate the effect of pinolenic acid on stimulated PGE2 levels in fibroblasts and on PMA stimulated sICAM-1 levels in fibroblasts;
- Figure 5 shows the effect of pinolenic acid on cornified envelope formation; and
- Figure 6 shows the effect of pinolenic acid on keratinocyte differentiation.

EXAMPLES

The following example demonstrates the anti-aging benefits of pinolenic acid.

It is know from our co-pending European application No. 99908956.8 that topical retinoic acid treatments can be used to cause upregulation of procollagen I and decorin in vivo.

To this end, the passages under the heading "Identification of procollagen I and decorin upregulation in skin in vivo following topical retinoic acid treatment for comparative purposes" in that application are incorporated herein in their entirety.

Example 1

Procedure For Measuring Procollagen-I and Decorin Synthesis In Human Dermal Fibroblasts

Preparation of Dermal Fibroblast Conditioned Medium

Primary human foreskin fibroblasts at passage 2 (P2) were seeded into 12-well plates at 10000 cells/cm^2 and maintained for 24 hours in an atmosphere of 5% carbon dioxide and 4% oxygen in Dulbeccos Modified Eagles Medium (DMEM) supplemented with 10% foetal calf serum. After this time the cells were washed with serum free DMEM and then incubated in fresh serum free DMEM for a further 60 hours. The fibroblast monolayers were then washed again with serum free DMEM. Test reagents and vehicle controls were added to the cells in triplicate in a final volume of 0.4ml/well fresh serum free DMEM and incubated for a further 24 hours. This fibroblast conditioned medium was either analysed immediately or snap frozen in liquid nitrogen and stored at -70°C for future analysis. The cells were then counted and data from the dot-blot analysis subsequently standardised to cell number.

Dot Blot Assay for Procollagen-I and Decorin Protein in Dermal Fibroblast Conditioned Medium

Samples of conditioned medium from dermal fibroblasts treated with vehicle (as a control) or test reagents were supplemented with 20mM dithiothreitol (1:10 dilution of 200mM stock solution) and 0.1% sodium dodecylsulphate (1:100 dilution of 10% stock solution), mixed well and then incubated at 75°C for 2 minutes. A standard for the assay was generated by serial dilution of neat fibroblast conditioned medium from fibroblasts seeded at 10000 cells/cm^2 in a 175cm^2 flask and maintained in serum free DMEM as described above.

Assay samples were subsequently applied in triplicate to a pre-wetted sheet of Immobilon-P transfer membrane using the 96-well Bio-Dot Apparatus from Bio-Rad as described in the manufacturers' guidelines. Approximately 200µl of medium was applied per well. The medium was allowed to filter through the membrane under gravity (30 minutes) after which the membrane was washed twice with PBS (200µl). These PBS washes were allowed to filter through the membrane under gravity (2x15 minutes). The Bio-Dot apparatus was then attached to a vacuum manifold and a third and final PBS wash carried out under suction. The apparatus was disassembled, the membrane removed and quickly cut as required before being placed in blocking buffer overnight at 4°C.

Membranes prepared for decorin analysis were blocked with 3% (w/v) BSA/ 0.1% (v/v) Tween 20 in PBS, whilst those for procollagen-I analysis were blocked with 5% (w/v) non fat dried milk powder/ 0.05% Tween 20 in PBS. The following day, the membranes were probed with 1:10000 dilution of primary antibodies to either human procollagen-I (MAB1912; rat monoclonal; Chemicon Int. Inc., Temecula, CA) or human decorin (rabbit polyclonal; Biogenesis) for 2 hours at room

temperature. The membranes were subsequently washed with TBS/ 0.05% Tween 20 (3 x 5 minutes) and then incubated with 1:1000 dilution of ^{125}I-conjugated anti-rat or anti-rabbit F(ab')2 fragments (Amersham) as required for 1 hour at room temperature.

Following this the Immobilon strips were again washed with TBS/Tween 20 (3 x 5 minutes) before being allowed to dry in air at room temperature. The dried membranes were wrapped in cellophane and exposed to a Molecular Dynamics storage phosphor screen for 16-18 hours. At the end of this time the exposed screen was scanned by a phosphorimager (Molecular Dynamics Phosphorimager SF) using ImageQuant™ software. Dot intensity was assessed by computer-assisted image analysis using the quantification tools in ImageQuant™, standardised to cell number and the effects of various test reagents on decorin and procollagen-I synthesis were determined relative to a vehicle treated control value of 100 arbitrary units.

Table 1 below indicates the effects of pinolenic acid on procollagen-I and decorin synthesis in human dermal fibroblasts, and the amounts in which it was applied. In order to normalise the results the effects of the test substance was determined relative to a vehicle treated control value of 100 arbitrary units. Figures 1 and 2 show the results for more data points graphically.

For comparison, a trial was performed with retinoic acid to assess its effect on decorin synthesis in human dermal fibroblasts. The concentrations of reagents used in the trials had no influence on cell viability.

Table 1 - The Effect on Procollagen-I and Decorin Synthesis by Pinolenic Acid

Treatment	Procollagen-I	Decorin
Control (Vehicle)	100	100
Pinolenic Acid (10μM)	148.2±2.3 (n=3)	119.0±6.9 (n=3)

The results in table 1 indicate that pinolenic acid significantly upregulates the synthesis of both procollagen-I and decorin in human dermal fibroblasts as compared to the control.

The level of decorin in skin is associated with improved condition and appearance of skin. Increasing the level of decorin in skin is important for controlled and correct deposition of collagen in skin which is associated with many skin benefits such as wrinkle effacement and dermal repair of photodamaged skin.

Example 2

This example measures the effect of pinolenic acid on reducing the inflammatory response of dermal fibroblasts.

Fibroblasts PGE_2 and ICAM Assay

Intracellular adhesion molecules (ICAM) and PEG_2 production by human skin fibroblasts can be induced by the inflammatory stimulus PMA (phorbal myristate acetate). PMA represents an external stressor which induces oxidative stress and

inflammatory responses in cells. This model is used to model inflammation *in vivo*.

Primary human foreskin fibroblasts at passage 2 (P2) were seeded into 96-well plates at 35,000 cells/well and maintained for 24 hours in an atmosphere of 5% carbon dioxide in Dulbeccos Modified Eagles Medium (DMEM) supplemented with 10% foetal calf serum. Pinolenic acid was added to fresh cell media (DMEM, supplemented with 10% foetal calf serum) in dimethylsulphoxide (ethanol, final concentration 1%) in triplicate and incubated for a further 24 hours. Phorbal myristate acetate (PMA), 10nM (Sigma) was added to the media and the cells incubated for a further 24 hours. The control did not contain any test compounds nor any PMA. The fibroblasts/media were then analysed as described below immediately or snap frozen in liquid nitrogen and stored at -70°C for future analysis. The cells were then counted and data from the dot-blot analysis subsequently standardised to cell number.

Prostaglandin E2 (PGE_2) assay: Volumes of 50 µl culture medium were taken for PGE_2 assay after gently shaking the culture plate. PGE_2 levels in the medium were determined with a Biotrak PGE_2 immunoassay kit (Amersham, UK). The assay is based on the competition between unlabelled PGE_2 in the sample and a fixed quantity of horseradish peroxidase labeled PGE_2 for a limited amount of fixed PGE_2 specific antibody. Concentrations of unlabelled sample PGE_2 are determined according to a standard curve which was obtained at the same time.

ICAM-1 assay: Media were discarded and cells washed with Dulbecco PBS. To the washed cells, 150 µl 0.1% Triton X-100 (Sigma) was added for 3 minutes to extract ICAM from cell membrane. The extracts were transferred to Eppendoff centrifuge tubes and centrifuged at 1000 g for 2 min to remove cell debris. A volume of 100 µl supernatant was used for ICAM assay. The soluble ICAM-1 was assessed with commercially available immunoenzymometric assay kit (R&D Systems). Concentrations of ICAM-1 in the samples were determined based on parallel running standard curve.

The results that were obtained from the PGE_2 and ICAM assay are summarised in table 2 below, and shown graphically in Figures 3 and 4.

Table 2 - Effects of pinolenic acid on PMA-induced ICAM and PGE_2 production in human skin fibroblasts

TREATMENT	N	ICAM (ng/ml)	PGE_2 (pg/ml)
Control	3	5.44 ± 0.53	1392.5 ± 411.1
PMA (10nM)-treated	3	9.46 ± 1.78	12800 ± 0.00*
PMA+PA 1ng/ml	3	7.80 ± 0.50	11652.7 ± 1243.2
PMA+PA 10ng/ml	3	7.45 ±0.32	8698.4 ± 2530.9
PMA+PA 100ng/ml	3	6.69 ± 0.18	4633 ± 1647.9
PMA+PA 1ug/ml	3	6.81 ± 0.56	3004 ± 961.0

* maxiumum level detectable by enzyme immunoassay

The above results show that challenging cells with an inflammatory stimulus such as PMA (Phorbol myristyl acetate) causes an increase in the inflammatory response as measured by prostaglandin E2 (PGE_2) production. Pinolenic acid, even

at the levels of 0.1μm, dramatically reduces the inflammatory response as measured by PGE_2 production. The results thus demonstrates that pinolenic acid has good anti-inflammatory activity.

The above results also demonstrate that challenging cells with PMA causes an increase in ICAM production. Pinolenic acid decreases the production of Intracellular adhesion molecule (ICAM), which is another marker of inflammation. These results thus further demonstrate that pinolenic acid has good anti-inflammatory action.

Example 3

Human foreskin keratinocytes at passage 3 (P3) were seeded into 96 well plates at 4000 cells/well in Dubleccos Modified Eagles Medium (DMEM), 0.03mM calcium. The cells were grown for 3 days prior to treatment. The treatment vehicle was DMSO. After 4 days of treatment, the cells were harvested and washed three times with 100μl phosphate buffered saline (PBS). The cells were then extracted in 1% Triton X100, 50mM Tris pH 8.0, 0.02mM Leupeptin, 0.02mM Pepstatin. 60μl/well of extract was then assayed for DNA concentration (ng/well), Pico Green DNA assay, Molecular Probes.

The cells were then washed in 200μl PBS, and then 100μl of 2% SDS, 20mM DTT was added to each well. The plates were then sealed with a Titertek plate sealer (ICN) and incubated at 60°C over night in an air tight damp environment (i.e. a sealed sandwich box lined with damp paper). The extract was then filtered through a PVDF transfer membrane (Bio-rad) under gravity using Dot-Blot apparatus (Bio-rad). The membrane is then washed in distilled water prior to silver

staining (Bio-rad Silver Stain kit). The stained dot blot membrane is then analysed using Phoretix array software (Phoretix International).

RESULTS

The results are shown in Table 3 below, and graphically in figures 5 and 6.

Table 3

	Cornified Envelope		DNA (ng/well)	
µM Pinolenic Acid	**Mean**	**SD**	**Mean**	**SD**
0	22831	6694	14.4	0.6
0.1	18828	1786	13.4	0.3
0.5	22236	3643	13.9	0.5
1	24860	4979	12.9	0.6
5	29606	9900	12.6	0.3
10	36579 (p=0.006*)	9286	12.6	0.3
20	34374 (p=0.018*)	5692	12.4	0.1

* relative to zero value. For the DNA values $P<0.001$.

The results show how cornified envelope production increased and DNA levels decreased in response to 0.1-20 µM application of pinolenic acid. This is indicative of enhanced keratinocyte differentiation, and suggests that pinolenic acid improves in situ skin barrier formation and resilience, and reduces trans-epidermal water loss and keratinocyte proliferation.

Example 4

The formulation below describes an oil in water cream suitable for the methods and uses according to the present invention. The percentages indicated are by weight of the composition.

	wt%
Mineral Oil	4
Pinolenic acid	1.15
Brij 56*	4
Alfol 16RD**	4
Triethanolamine	0.75
Butane-1,3-diol	3
Xanthan gum	0.3
Perfume	qs
Butylated hydroxy toluene	0.01
Water	To 100

*Brij 56 is cetyl alcohol POE (10)

** Alfol 16RD is cetyl alcohol

Example 5

The formulation below describes an emulsion cream according to the present invention.

FULL CHEMICAL NAME OR CTFA NAME	TRADE NAME	WT. %
Pinolenic acid		2.0
disodium EDTA	Sequesterene Na2	0.05
Magnesium aluminium silicate	Veegum Ultra	0.6
methyl paraben	Methyl Paraben	0.15
Simethicone	DC Antifoam Emulsion	0.01
butylene glycol 1,3	Butylene Glycol 1,3	3.0
Hydroxyethylcellulose	Natrosol 250HHR	0.5
Glycerine, USP	Glycerine USP	2.0
xanthan gum	Keltrol 1000	0.2
Triethanolamine	Triethanolamine (99%)	1.2
stearic acid	Pristerene 4911	3.0
propyl paraben NF	Propylparaben NF	0.1
glyceryl hydrostearate	Naturechem GMHS	1.5
stearyl alcohol	Lanette 18 DEO	1.5
Isostearyl palmitate	Protachem ISP	6.0
C12-15 alcohols octanoate	Hetester FAO	3.0
Dimethicone	Silicone Fluid 200 (50cts)	1.0
Cholesterol NF	Cholesterol NF	0.5
sorbitan stearate	Sorbitan Stearate	1.0
Butylated hydroxytoluene	Embanox BHT	0.05
Tocopheryl acetate	Vitamin E Acetate	0.1
PEG-100 stearate	Myrj 59	2.0
sodium stearoyl lactylate	Pationic SSL	0.5
Hydroxycaprylic acid	Hydroxycaprylic Acid	0.1
retinyl palmitate	Vitamin A Palmitate	0.06
alpha-bisabolol	Alpha-bisabolol	0.2
water, DI		q.s. to 100

Both the above topical compositions of examples 4 and 5 provide an effective cosmetic treatment to improve the appearance of wrinkled, aged, photo-damaged, and/or irritated skin, when applied to skin that has deteriorated through the aging or photoaging or when applied to youthful skin to help prevent or delay such deteriorative changes. The compositions can be processed in conventional manner.

CLAIMS

1. A topical composition comprising:

   (a) an effective amount of pinolenic acid; and
   (b) a dermatologically acceptable vehicle.

2. A topical composition according to claim 1, wherein the pinolenic acid is present in the topical composition at a level of 0.1 to 5% by weight of the topical composition.

3. A cosmetic method of providing at least one skin care benefit selected from: treating/preventing wrinkling, sagging, aged and/or photodamaged skin; boosting collagen deposition in skin, boosting decorin production in skin, enhancing tissue repair; improving skin condition and resilience through enhanced barrier formation; treating dry and flaky skin; reduced hyperproliferation; soothing irritated, red and/or sensitive skin; and improving skin texture, smoothness and/or firmness; the method comprising applying to the skin a topical composition comprising pinolenic acid and/or derivatives thereof.

4. Use of pinolenic acid and/or derivatives thereof in a topical composition for providing at least one skin care benefit selected from treating/preventing wrinkling, sagging, aged and/or photodamaged skin; boosting collagen deposition in skin, boosting decorin production in skin, enhancing tissue repair; improving skin condition and resilience through enhanced barrier formation; treating

dry and flaky skin; reduced hyperproliferation; soothing irritated, red and/or sensitive skin; improving skin texture, smoothness and/or firmness.

5. Use of pinolenic acid and/or derivatives thereof in the prparation of a topical composition for providing at least one skin care benefit selected from treating/preventing wrinkling, sagging, aged and/or photodamaged skin; boosting collagen deposition in skin, boosting decorin production in skin, enhancing tissue repair; improving skin condition and resilience through enhanced barrier formation; treating dry and flaky skin; reduced hyperproliferation; soothing irritated, red and/or sensitive skin; and improving skin texture, smoothness and/or firmness.

6. Pinolenic acid and/or derivatives thereof for use in treating/preventing wrinkling, sagging, aged and/or photodamaged skin; boosting collagen deposition in skin, boosting decorin production in skin, enhancing tissue repair; improving skin condition and resilience through enhanced barrier formation; treating dry and flaky skin; reduced hyperproliferation; soothing irritated, red and/or sensitive skin; and improving skin texture, smoothness and/or firmness.

7. A topical composition comprising: (i) an effective amount of pinolenic acid and/or derivatives thereof; and (ii) a cosmetically and/or dermatologically acceptable vehicle for use in treating/preventing wrinkling, sagging, aged and/or photodamaged skin; boosting collagen deposition in skin, boosting decorin production in skin, enhancing tissue repair; improving skin condition and resilience

through enhanced barrier formation; treating dry and flaky skin; reduced hyperproliferation; soothing irritated, red and/or sensitive skin; and improving skin texture, smoothness and/or firmness.

Fig.1.

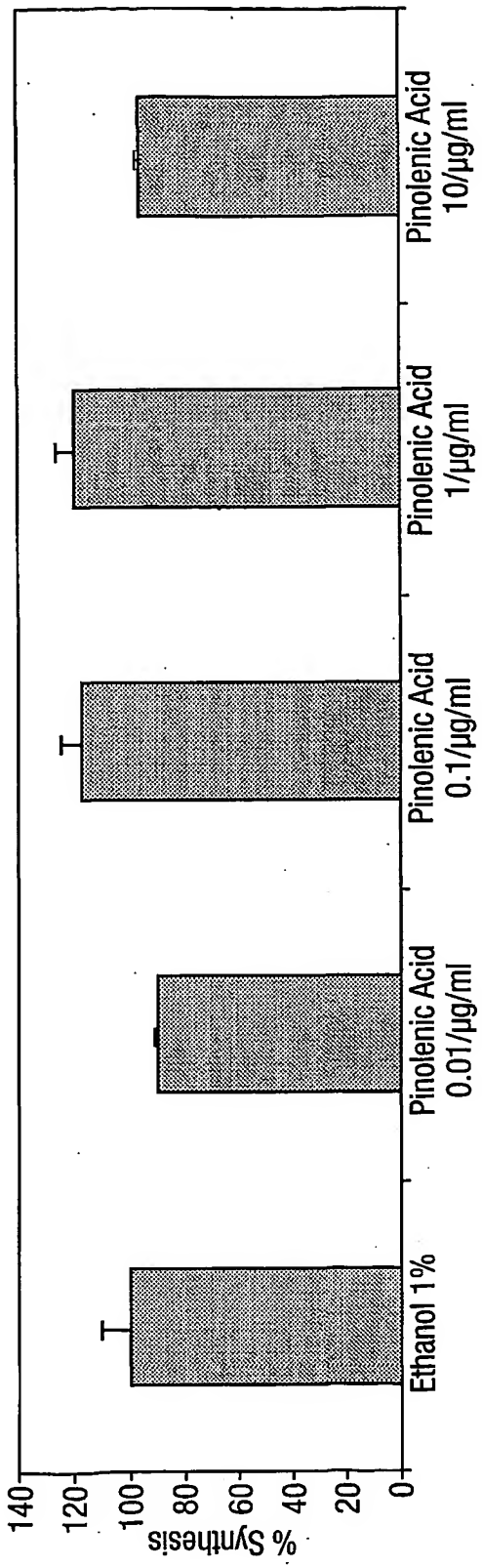
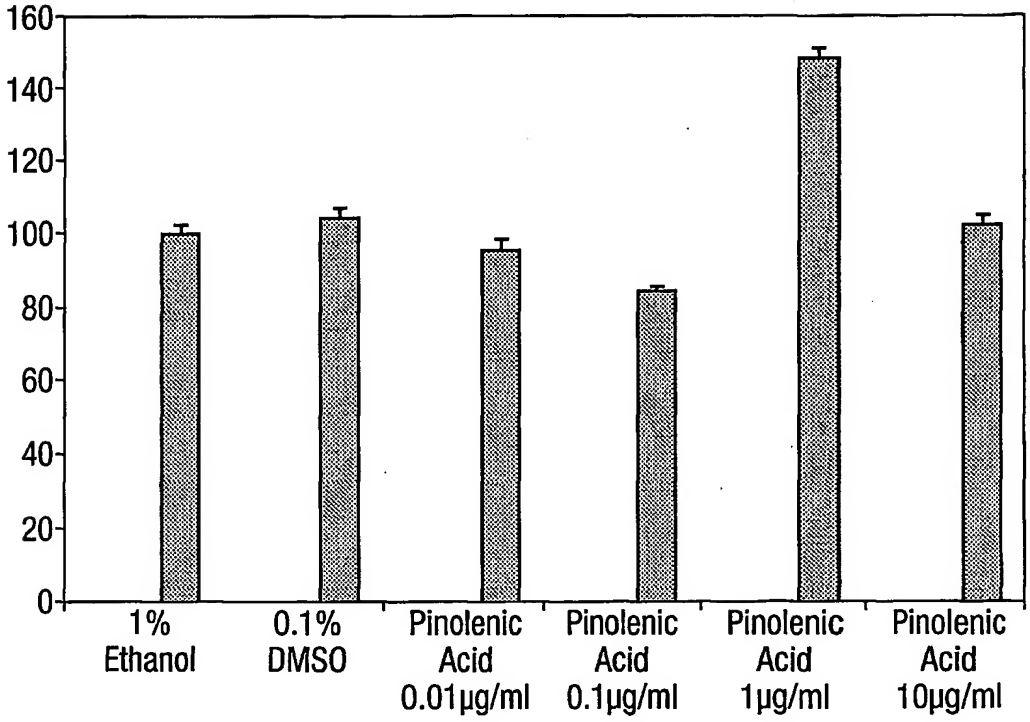

% Synthesis
0
20
40
60
80
100
120
140
Ethanol 1%
Pinolenic Acid 0.01/µg/ml
Pinolenic Acid 0.1/µg/ml
Pinolenic Acid 1/µg/ml
Pinolenic Acid 10/µg/ml

Fig.2.


160
140
120
100
80
60
40
20
0
1% Ethanol
0.1% DMSO
Pinolenic Acid 0.01µg/ml
Pinolenic Acid 0.1µg/ml
Pinolenic Acid 1µg/ml
Pinolenic Acid 10µg/ml

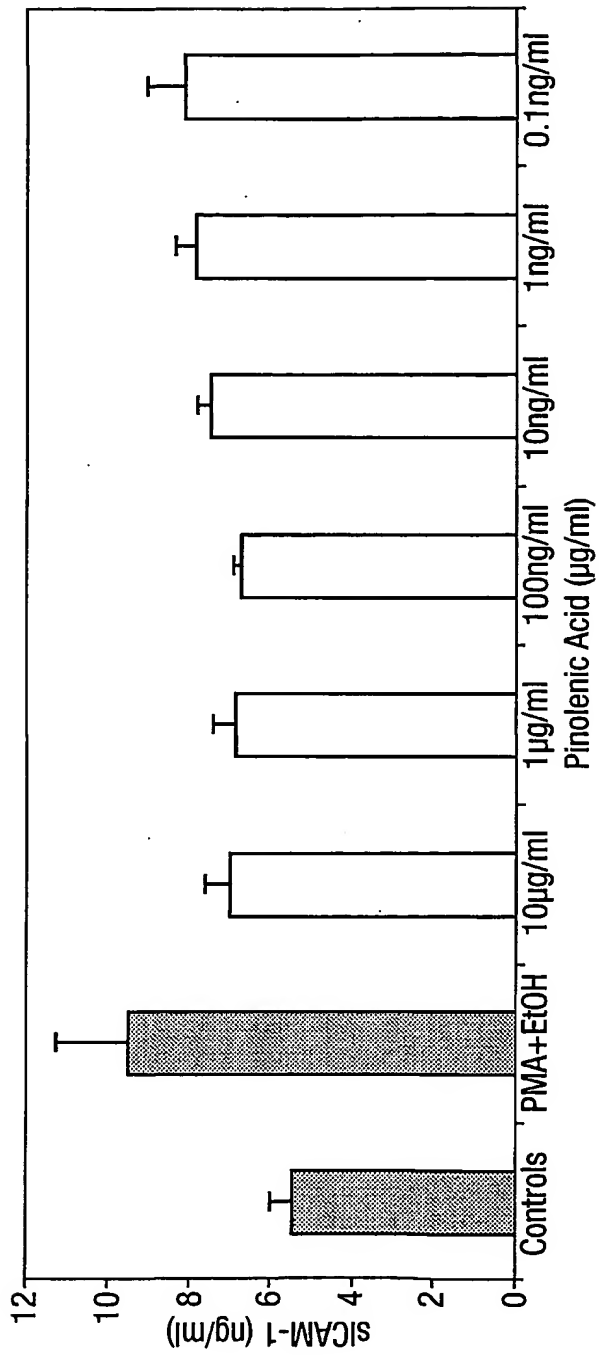

sICAM-1 (ng/ml)
0
2
4
6
8
10
12
Controls
PMA+EtOH
10µg/ml
1µg/ml
100ng/ml
10ng/ml
1ng/ml
0.1ng/ml
Pinolenic Acid (µg/ml)

Fig.3.

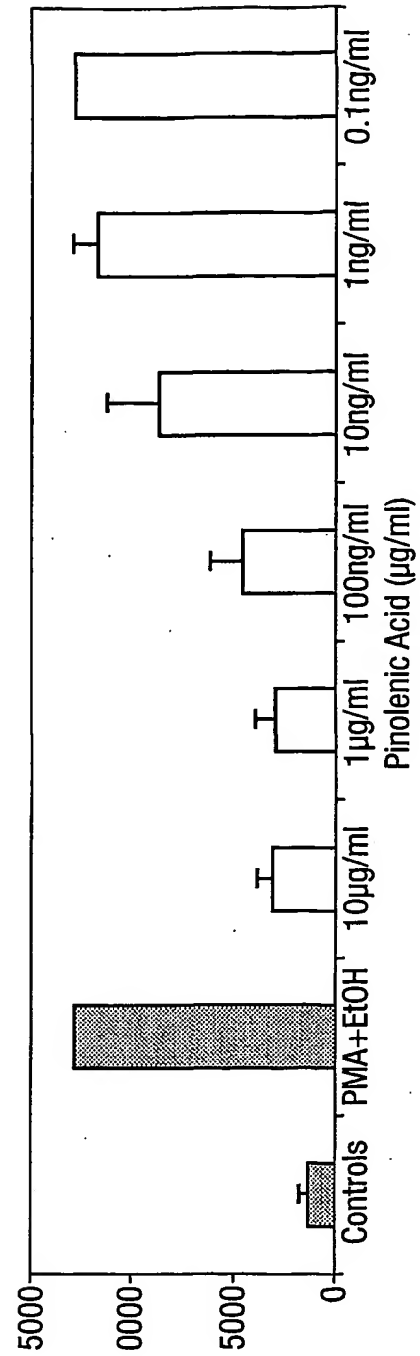

PGE2 (pg/ml)
0
5000
10000
15000
Controls
PMA+EtOH
10µg/ml
1µg/ml
100ng/ml
10ng/ml
1ng/ml
0.1ng/ml
Pinolenic Acid (µg/ml)

Fig.4.

Fig.5.

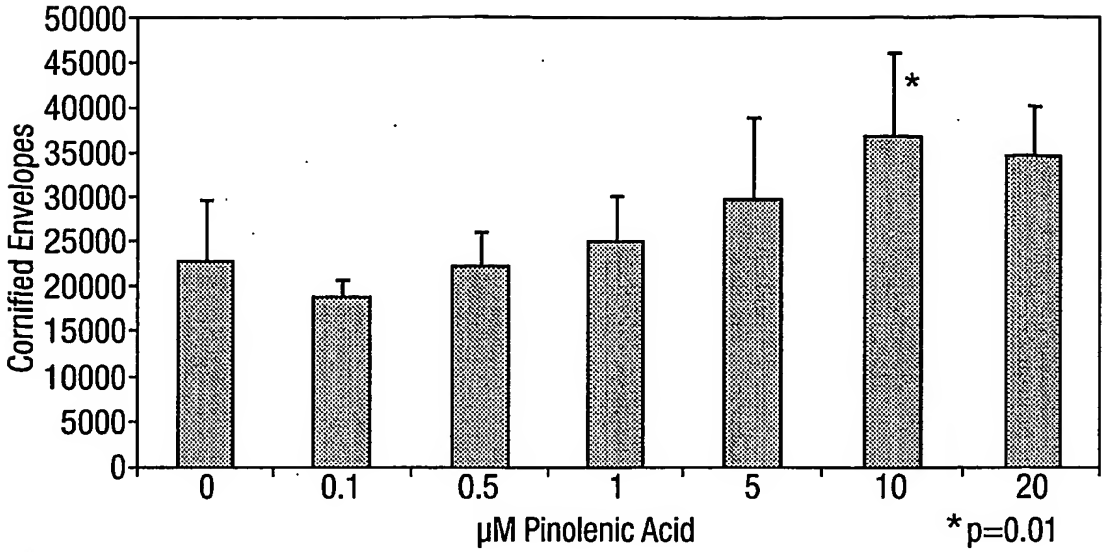
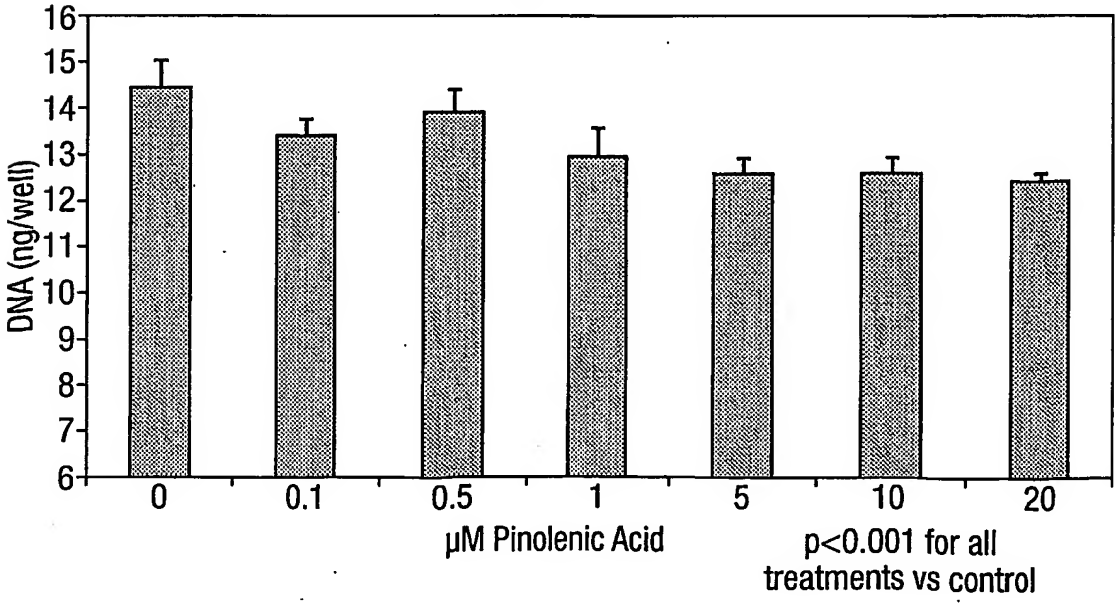

Cornified Envelopes
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
*
0
0.1
0.5
1
5
10
20
µM Pinolenic Acid
*p=0.01

Fig.6.


DNA (ng/well)
16
15
14
13
12
11
10
9
8
7
6
0
0.1
0.5
1
5
10
20
µM Pinolenic Acid
p<0.001 for all
treatments vs control

INTERNATIONAL SEARCH REPORT

Int. - al Application No
PCT/EP 01/13039

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K7/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 756 465 A (D A NOSTE) 5 June 1998 (1998-06-05) page 4, line 3-24; claims 1-11	1-7
X,P	WO 01 08653 A (UNILEVER PLC ;LEVER HINDUSTAN LTD (IN); UNILEVER NV (NL)) 8 February 2001 (2001-02-08) page 10, line 23 -page 11, line 2; claims 1-8 page 12, line 7-11	1-9
X,P	EP 1 088 552 A (UNILEVER PLC ;UNILEVER NV (NL)) 4 April 2001 (2001-04-04) page 2, line 37-46; claim 1	1,2,6,7

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2002	11/04/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lindner, A

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INTERNATIONAL SEARCH REPORT

Int[illegible]al Application No
PCT/EP 01/13039

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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